Spatiotemporal organization of Ca^{2+} dynamics: a modeling-based approach

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Calcium is a ubiquitous second messenger that mediates vital physiological responses such as fertilization, secretion, gene expression, or apoptosis. Given this variety of processes mediated by Ca^{2+} , these signals are highly organized both in time and space to ensure reliability and specificity. This review deals with the spatiotemporal organization of the Ca^{2+} signaling pathway in electrically nonexcitable cells in which InsP_3 receptors are by far the most important Ca^{2+} channels. We focus on the aspects of this highly regulated dynamical system for which an interplay between experiments and modeling is particularly fruitful. In particular, the importance of the relative densities of the different InsP_3 receptor subtypes will be discussed on the basis of a modeling approach linking the steady-state behaviors of these channels in electrophysiological experiments with their behavior in a cellular environment. Also, the interplay between InsP_3 metabolism and Ca^{2+} oscillations will be considered. Finally, we discuss the relationships between stochastic openings of the Ca^{2+} releasing channels at the microscopic level and the coordinated, regular behavior observed at the whole cell level on the basis of a combined experimental and modeling approach. [DOI: 10.2976/1.3385660]

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Calcium is one of the most widespread second messenger in cellular biology. It mediates vital physiological responses as, for example, fertilization, secretion, gene expression, or apoptosis (Berridge *et al.*, 2003; Combettes *et al.*, 2004). Importantly, these Ca^{2+} signals are highly organized in time and space (Dupont *et al.* 2007). When cells are activated, particularly through receptors leading to the synthesis of inositol 1,4,5-trisphosphate (InsP_3), the level of Ca^{2+} often oscillates with a frequency that varies with agonist concentration. These frequency-encoded Ca^{2+} pulses then convey information as to the nature and the extent of the stimulated physiological response. Moreover, each Ca^{2+} pulse spreads throughout the cytosol in the form of a wave. It is now well established that InsP_3 -induced Ca^{2+} oscillations originate from the repetitive opening of InsP_3 receptors (InsP_3R), which

are Ca^{2+} channels embedded in the membrane of the endoplasmic reticulum (ER) that behaves as a huge Ca^{2+} store. When open, the InsP_3R allows for a flux of Ca^{2+} down its electrochemical gradient. The concentration of Ca^{2+} in the ER $[[\text{Ca}^{2+}]_{\text{ER}} \approx 500 \mu\text{M}]$, see Chatton *et al.* (1995) and Meldolesi and Pozzan (1998) is indeed considerably higher than in the cytosol $[[\text{Ca}^{2+}]_i \approx 0.1 \mu\text{M}]$. Cytosolic Ca^{2+} also regulates the Ca^{2+} releasing activity of this channel. InsP_3 and Ca^{2+} thus act as coagonists of the InsP_3R . Intermediate levels of cytosolic Ca^{2+} activate Ca^{2+} release from the ER while high levels of Ca^{2+} ultimately lead to channel closing (Bezprozvanny *et al.*, 1991). Models indicate that this biphasic regulation of InsP_3 receptors by Ca^{2+} can explain both oscillations and waves much resembling experimental observations.

Calcium release is initiated by inositol 1,4,5-trisphosphate (InsP_3),

generated in response to the external stimulus through a well-characterized signaling cascade (Berridge, 1993). Oscillations are then maintained as long as InsP_3 remains elevated in the cytoplasm. As expected, oscillations in $[\text{Ca}^{2+}]_{\text{ER}}$ are observed in antiphase to $[\text{Ca}^{2+}]_i$ oscillations (Ishii *et al.*, 2006). Although Ca^{2+} oscillations generally take the form of regular spikes, more complex Ca^{2+} oscillations have also been observed. In particular, bursting behavior similar to that displayed by electrically excitable cells has been reported. One of the best known example of Ca^{2+} bursting is the complex oscillatory pattern seen in hepatocytes after stimulation by specific agonists such as cAMP or ATP (Dixon *et al.*, 1993; Green *et al.*, 1997). The mechanism underlying this type of Ca^{2+} oscillations remains poorly understood from an experimental point of view and has mainly been investigated by several theoretical approaches (Kummer *et al.*, 2000; Marhl *et al.*, 2000).

It is often assumed that the potency of the cell to control so diverse physiological processes with a compound as simple as Ca^{2+} ions results from the large versatility of the signal-induced Ca^{2+} changes. The existence of different InsP_3R isoforms is probably an important factor allowing such a diversity of responses [see Taylor *et al.* (1999) and Vermassen *et al.* (2004) for reviews]. The interplay between InsP_3 metabolism and Ca^{2+} signaling is another factor that can modulate signal-induced Ca^{2+} rises depending on the cell type or other internal factors such as the specific cytoplasmic locus or the developmental state of the cell (Dupont and Erneux, 1997). In this review, we will first give a brief outline about models for Ca^{2+} oscillations based on the dynamics of the InsP_3R (Deterministic models for Ca^{2+} oscillations section). After that, we will emphasize how this basic oscillatory mechanism can be affected either by differences at the regulatory level due to specific receptor isoforms (Impact of the InsP_3 receptor isoform on Ca^{2+} oscillations section), or by interplay with InsP_3 metabolism (Interplay between Ca^{2+} oscillations and InsP_3 metabolism section).

Besides the observation of global Ca^{2+} signals, an impressive number of experimental studies have been devoted to the imaging of the Ca^{2+} releasing activity of a small number of InsP_3 receptors *in vivo*. This activity can occur either spontaneously or at submaximal InsP_3 concentrations. As can be expected, these events appear to be stochastic. Their amplitude and the interval between them vary significantly under the same conditions, allowing only for a statistical description. The smallest observed events, called blips, provoke a rise in cytosolic Ca^{2+} of about 40 nM and last in average 70 ms. These data correspond to successive openings of a single InsP_3 receptor in the cytosol, which is possible due to the poor diffusing properties of Ca^{2+} in the cytoplasm allowing for rapid rebinding of Ca^{2+} on the channels activating sites, as indicated by quantitative models (Swillens *et al.*, 1998; Shuai and Jung, 2002; Thul and Falcke, 2004; Nguyen *et al.*, 2005; Cooper *et al.*, 2009). Alternatively, the openings of one InsP_3R may trigger other InsP_3Rs within a cluster to generate slightly larger Ca^{2+} increases known as

Ca^{2+} puffs. The rise in Ca^{2+} is then of about 200 nM and lasts ~ 300 ms. Blips and puffs have been extensively studied in HeLa cells (Thomas *et al.*, 2000; Bootman *et al.*, 2002) and *Xenopus* oocytes (Marchant *et al.*, 1999; Smith and Parker, 2009). These elementary events have been modeled using a stochastic description of the dynamics of the InsP_3R (Swillens *et al.*, 1999; Williams *et al.*, 2008; Smith *et al.*, 2009). Given that (irregular) Ca^{2+} puffs and (much more regular) oscillations can be observed in the same cell for different levels of stimulus, the study of Ca^{2+} dynamics offers the fascinating possibility to study the transition from a stochastic to a deterministic regime. This transition corresponds to the emergence of a macroscopic self-organized system, which is a central concern in biology (Nicolis and Prigogine, 1977). The current development in this field, in relation with Ca^{2+} dynamics, will be briefly reviewed in the Stochastic aspects of Ca^{2+} oscillations section.

DETERMINISTIC MODELS FOR Ca^{2+} OSCILLATIONS

Most models for InsP_3 -induced Ca^{2+} oscillations rely on a mathematical description of both the Ca^{2+} transfer processes and the regulatory mechanisms known to play an essential role in this periodic behavior. In particular, the rapid activation-slow inhibition of the InsP_3R by cytosolic Ca^{2+} clearly plays a crucial role in the onset of oscillations (Li and Rinzel, 1994). Thus, models need to reproduce this regulation, and the associated well-known bell-shaped curve for the stationary open probability of the InsP_3R as a function of cytosolic Ca^{2+} (Bezprozvanny *et al.*, 1991). One of these models is schematized in Fig. 1. As shown in the left panel, it is based on the mutually exclusive activation-inhibition of the InsP_3 receptor by Ca^{2+} . It is assumed that InsP_3 and Ca^{2+} -mediated activation are instantaneous, whereas Ca^{2+} -induced inhibition develops slowly. The binding of Ca^{2+} to the activating and inhibitory sites are supposed to be mutually exclusive. If activation and inhibition are considered as cooperative processes characterized by Hill coefficients n_a and n_i , respectively, the change in the fraction of inactive receptors (R_i) obeys the following equation [see Dupont and Swillens (1996) for a detailed description of the equations]:

$$\frac{dR_i}{dt} = k_+ C^{n_i} \frac{1 - R_i}{1 + \left(\frac{C}{K_{\text{act}}}\right)^{n_a}} - k_- R_i, \quad (1)$$

where k_+ and k_- are the kinetic constants of Ca^{2+} association to and dissociation from the inhibitory Ca^{2+} binding site of the receptor, C represents the concentration of cytosolic Ca^{2+} , and K_{act} is the dissociation constant of Ca^{2+} binding to the activating Ca^{2+} binding site of the InsP_3R . The time evolution of the concentration of cytosolic Ca^{2+} is given by

$$\frac{dC}{dt} = k_1(b + \text{IR}_a)[C_{\text{tot}} - C(\alpha + 1)] - V_{\text{MP}} \frac{C^2}{K_p^2 + C^2} \quad (2)$$

in which IR_a represents the fraction of active (i.e., open) InsP_3R and is given by

Figure 1. Schematic representation of a model for Ca²⁺ oscillations based on the regulatory properties of the InsP₃ receptor/Ca²⁺ channel. Oscillations occur through the periodic exchange of Ca²⁺ between the cytoplasm and the ER. The left panel represents the mechanism by which InsP₃ and Ca²⁺ regulate Ca²⁺ release through the InsP₃ receptor with a fast activation by Ca²⁺ and InsP₃ and a slower inhibition by Ca²⁺ at large concentrations. The model assumes that binding of Ca²⁺ to the activating and inhibitory sites are mutually exclusive. The only open state of the receptor/channel is IR_a in which InsP₃ is bound as well as Ca²⁺ on the activating site. Green indicates that the molecule/ion has a positive effect on the activity of the channel while red refers to a negative effect. R: InsP₃ receptor with no ligand, IR: InsP₃ receptor with InsP₃ bound, R_a: InsP₃ receptor with Ca²⁺ bound to the activating site, IR_a: InsP₃ receptor with InsP₃ bound and Ca²⁺ bound to the activating site, R_i: InsP₃ receptor with Ca²⁺ bound to the inhibitory site, and IR_i: InsP₃ receptor with InsP₃ bound and Ca²⁺ bound to the inhibiting site.

$$IR_a = IR_{\text{able}} \frac{C^{na}}{K_{\text{act}}^{na} + C^{na}}, \quad (3)$$

where IR_{able} is the fraction of receptors that can be activated (“activatable”). This fraction, which represents the fraction of noninhibited receptors bound to InsP₃, can also be evaluated from an algebraic equation.

$$IR_{\text{able}} = (1 - R_i) \frac{IP^3}{K_{IP}^3 + IP^3}. \quad (4)$$

IP stands for the intracellular concentration in InsP₃. k_1 is the kinetic constant governing the flux of Ca²⁺ from the lumen into the cytosol and k_1b represents the basal flux in the absence of InsP₃. K_{IP} is the dissociation constant of InsP₃ binding to its receptor. In agreement with experimental results, activation by InsP₃ is taken as a cooperative process with a Hill coefficient equal to 3. C_{tot} represents the total intracellular concentration of free Ca²⁺ (not bound to buffers). α is a factor defined as the ratio between the volume of the ER and the volume of the cytosol. As total Ca²⁺ is assumed to remain constant (the model does not consider possible Ca²⁺ exchanges with the extracellular medium), the term between brackets in Eq. (2) represents the difference in Ca²⁺ concentration between the ER and the cytosol. The second term of the right-hand side of Eq. (2) stands for Ca²⁺ pumping back from the cytosol into the ER, a process which is known to be mediated by SERCA pumps, which are well-characterized ATPases (Lyttton *et al.*, 1992).

Various mathematical models based on this core oscillatory mechanism have been proposed (see Thul *et al.* (2008) for a recent review). While, as in the example above, some models use an empirical description of the successive positive and negative feedbacks leading to oscillations (Atri *et al.*, 1993; Dupont and Swillens, 1996), mechanistic models describing in a more detailed way the transitions between the states of the receptor channel have also been proposed (De Young and Keizer, 1992; Tang *et al.*, 1996). As these models describe the evolution of the various states of the receptors, they usually contain a large number of variables. However, quasi-steady-state assumptions on some receptor states can drastically simplify the dynamical description and reduce the system to a two-variable system whose equations are similar to the Hodgkin–Huxley equations (Li and Rinzel, 1994). It should be noted that besides InsP₃R regulation, some models take into account other phenomena related to InsP₃ and/or Ca²⁺ dynamics that markedly influence Ca²⁺ oscillations and waves in some cell types. Thus, Ca²⁺ feedbacks at the level of InsP₃ synthesis or metabolism into InsP₂ or InsP₄ can markedly influence the time evolution of Ca²⁺ and InsP₃ (Meyer and Stryer, 1991; Dupont and Erneux, 1997; Politi *et al.*, 2006). Because Ca²⁺ is electrically charged, ER membrane potential oscillations can also play a role (Marhl *et al.*, 2000). The fact that Ca²⁺ enters the cytosol both from the ER and from the external medium makes the understanding of the observed Ca²⁺ dynamics significantly more difficult and has also been considered in some models (Sneyd *et al.*, 2004). Finally, Ca²⁺-activated protein kinase C (Kummer *et al.*, 2000; Kang and Othmer, 2007) or phosphorylation of the InsP₃R (LeBeau *et al.*, 1999) have been shown to affect the rate of InsP₃ synthesis or Ca²⁺ dynamics after stimulation of some types of phospholipase (PLC)-linked external receptors.

IMPACT OF THE InsP₃ RECEPTOR ISOFORM ON Ca²⁺ OSCILLATIONS

Three isoforms of the InsP₃R have been identified (InsP₃R1, InsP₃R2, and InsP₃R3) and their levels of expression are largely tissue specific. Experiments where the levels of expression of these isoforms have been modified (overexpress or knock-down) clearly indicate that their proportions significantly affect the time course of cytosolic Ca²⁺ concentration (Miyakawa *et al.*, 1999; Morel *et al.*, 2003; Hattori *et al.*, 2004). The three receptor subtypes are distributed inhomogeneously throughout the cytoplasm. For example, in hepatocytes, InsP₃-induced Ca²⁺ signals begin sooner in the apical region where InsP₃R2 are concentrated (Pusl and Nathanson, 2004). The various ways of regulating Ca²⁺ dynamics lead to different functions. For example, the type 3 isoform is specifically involved in the induction of apoptosis by preferentially transmitting Ca²⁺ signals into mitochondria (Mendes *et al.*, 2005). The level of expression of the different subtypes is also developmental and conditions specific. For example, in cardiomyocytes, the

levels of type 1 and 2 receptors are increased, but differently, by stressors such as cold exposure and hypoxia (Krizanová *et al.*, 2008).

Although all the InsP_3R subtypes display similar ion permeation properties, they differ significantly in their regulatory properties due to different interactions with accessory proteins, various modes of regulation by ATP or phosphorylation (Taylor and Laude, 2002; Patterson *et al.*, 2004). The exact nature of these differences, as well as their molecular origin, remains controversial. It is clear that they are differently regulated by Ca^{2+} and InsP_3 . In particular, different open probabilities at steady-state have been reported. Although consistent experimental data are lacking, the classical bell-shaped curve most probably corresponds to type 2 InsP_3R . This curve can be explained by a change in the effect of cytosolic Ca^{2+} on channel activity, which passes from activating to inhibitory when increasing Ca^{2+} concentration. Another subtype, presumably type 3, displays similar activation at low Ca^{2+} concentration but inhibition occurs for higher Ca^{2+} levels in the range of a few micromolars. Finally, a displacement of the bell-shaped curve with InsP_3 has also been reported. In this case, which corresponds to the $\text{InsP}_3\text{R}1$, the maximum of the bell-shaped curve shifts to the right when increasing the InsP_3 concentration (Kaftan *et al.*, 1997; Moraru *et al.*, 1999). The differences among the subtypes concerning regulation by InsP_3 are much less controversial: type 2 has the greatest affinity for InsP_3 , followed by types 1 and 3 successively (Vermassen *et al.*, 2004).

On the basis of the observed steady-state open probabilities, modeling has allowed to link the changes in the overall Ca^{2+} dynamics observed after modification of the levels of expression of the various subtypes to their different regulations by InsP_3 and Ca^{2+} (Dupont and Combettes, 2006). Thus, type 2 receptor, which shows the sharpest dependence on cytosolic Ca^{2+} and has the highest affinity for InsP_3 is the main oscillatory unit, as shown in DT40 cells (Miyakawa *et al.*, 1999) and myocytes (Morel *et al.*, 2003). Stimulation of type 1 receptor can also lead to oscillations but most often damped rather than sustained (Miyakawa *et al.*, 1999; Hattori *et al.*, 2004). In contrast, type 3 receptor tends to suppress oscillations. This surprising result is due to the fact that $\text{InsP}_3\text{R}3$, which are not inhibited at the Ca^{2+} level reached during oscillations, provide a constant flux of Ca^{2+} without providing the negative feedback necessary for oscillations to occur. Thus, when all three InsP_3 receptor subtypes are present, type 3 receptor constantly releases Ca^{2+} , which “pushes” types 1 and 2 receptors into the regime where cytosolic Ca^{2+} inhibits further Ca^{2+} release. Modeling however predicts that this suppression of oscillations occurs only at relatively high receptor density, as is illustrated in Fig. 2 (left panels). In a cell type where the total receptor density ($\text{InsP}_3\text{R}1 + \text{InsP}_3\text{R}2 + \text{InsP}_3\text{R}3$) is low (Fig. 2, right panels), the constant Ca^{2+} flux provided by type 3 receptors could activate types 1 and 2 and thereby favor Ca^{2+} oscillations (Dupont and Combettes, 2006). This prediction might be related to the obser-

vation performed in pancreatic acinar cells that the addition of both $\text{InsP}_3\text{R}2$ and $\text{InsP}_3\text{R}3$ support Ach and CCK-induced Ca^{2+} oscillations (Futatsugi *et al.*, 2005).

InsP_3 receptors mostly exist in the form of tetramers. These functional channels are composed of subunits of the same (homotetramers) or different isoforms (heterotetramers), which adds another level of complexity to the present modeling approach. In the same line, the fact that InsP_3Rs form macromolecular signaling complexes with other proteins such as calmodulin-kinase II or phosphatases 1 and 2A should not be overlooked (Mikoshiba, 2007). These accessory proteins indeed regulate channel opening and thereby affect Ca^{2+} signaling. Little is known about the possibly distinct abilities of the different isoforms of the InsP_3Rs to form these signaling complexes, as most studies have been performed in cerebellar tissues, which mostly express type 1 InsP_3R .

INTERPLAY BETWEEN Ca^{2+} OSCILLATIONS AND InsP_3 METABOLISM

Membrane-bound PLC is responsible for InsP_3 synthesis via hydrolysis of PIP_2 . This enzyme possesses several isoforms (β , γ , δ , ϵ , and ζ), differing by the signaling pathway in which they are involved and by their Ca^{2+} sensitivity. $\text{PLC}\beta$ are activated by protein-G coupled receptors, $\text{PLC}\gamma$ by tyrosine kinases, $\text{PLC}\delta$ by PIP_2 and Ca^{2+} , $\text{PLC}\epsilon$ by Ras, and $\text{PLC}\zeta$ by Ca^{2+} . Theoretically, stimulation of PLC activity by Ca^{2+} in the appropriate concentration range can lead to concomitant Ca^{2+} and InsP_3 oscillations with a period that depends on the rate of InsP_3 synthesis by PLC (Meyer and Stryer, 1991). Concerning its degradation, InsP_3 can be either dephosphorylated by the $\text{Ins}(1,4,5)\text{P}_3$ 5-phosphatase to yield $\text{Ins}(1,4)$ bisphosphate or phosphorylated by the $\text{Ins}(1,4,5)\text{P}_3$ 3-kinase into $\text{Ins}(1,3,4,5)$ tetrakisphosphate (Shears, 1992). The 3-kinase is stimulated by an increase in Ca^{2+} as binding of Ca^{2+} /calmodulin enhances its activity. The stimulation factor varies from 2 to 10 depending on the isoform (Takazawa *et al.*, 1990; Sims and Allbritton, 1998). Importantly, the product of InsP_3 phosphorylation by 3-kinase, InsP_4 , is a competitive inhibitor of the other InsP_3 -metabolizing enzyme, the 5-phosphatase (see Fig. 3). Much care should thus be taken when interpreting experiments where the levels of 3-kinase have been modified, as these changes are, at least in part, counteracted by the changes in activity of 5-phosphatase. As expected intuitively, the incorporation of such a regulation in a mathematical model can also lead to InsP_3 oscillations as each Ca^{2+} spike provokes an enhanced degradation of InsP_3 (Dupont and Erneux, 1997). However, in contrast to the stimulation of PLC activity by Ca^{2+} , in this case, InsP_3 oscillations passively follow Ca^{2+} oscillations (i.e., this regulation cannot on its own give rise to sustained oscillations and the peak of InsP_3 slightly follows that of Ca^{2+}). Before turning to the question of the relevance of these regulations of InsP_3 metabolism by Ca^{2+} in real cells, it should be mentioned that it is not possible to exclude that InsP_3 oscillations result from other regulatory influences on InsP_3 synthesis, more specifically at a step located upstream of

Figure 2. Simulations of the possibly antagonistic effects of the expression of InsP_3 receptors of type 3 on Ca^{2+} oscillations. If the total density of other types of InsP_3 receptors is high, the addition of a small amount of InsP_3 receptors of type 3 tends to suppress Ca^{2+} oscillations while it can transform damped Ca^{2+} oscillations into sustained ones if the initial density of receptors is low. For equations and parameter values, see [Dupont and Combettes \(2006\)](#).

PLC activation. For hepatocytes, for example, a specific regulation at the level of the $G\alpha$ subunit of the G-protein activation cascade leading to $\text{PLC}\beta$ activation might play such a role ([Kummer *et al.*, 2000](#)). Also, Ca^{2+} and InsP_3 oscillations induced by the activation of mGluR5 receptors rely on a cyclical inactivation of these receptors due to the phosphorylation by PKC ([Nash *et al.*, 2002](#)).

Although it is since long known that InsP_3 3-kinase is activated by Ca^{2+} , it took some time to clearly state that this regulation leads to InsP_3 oscillations. However, based on a careful quantitative evaluation of the kinetics of InsP_3 metabolism by the kinase and the phosphatase, the modeling suggests that InsP_3 oscillations resulting from this regulation have a tiny amplitude ([Dupont and Erneux, 1997](#)). Cellular conditions are indeed such that the major part of the InsP_3 pool is catabolized by 5-phosphatase. This conclusion holds with the observation performed in CHO cells that overexpression of 5-phosphatase clearly abolishes any Ca^{2+} oscillatory activity in response to

stimulation while a similar 3-kinase overexpression has a negligible effect on internal Ca^{2+} mobilization ([De Smedt *et al.*, 1997](#)).

Despite this observation, one might speculate that in other cell types, the 3-kinase might play a more important role, and, more especially, allow for a rather long time interval between two Ca^{2+} spikes. If each Ca^{2+} spike provokes the catabolism of a significant amount of InsP_3 , it could take some time for InsP_3 to reach a level sufficient to trigger Ca^{2+} release. In this view, the long time interval between two successive Ca^{2+} spikes would correspond to the time necessary to rebuild the level of InsP_3 necessary to activate Ca^{2+} release through the receptor. This possibility has been indirectly tested in hepatocytes ([Dupont *et al.*, 2003](#)). The strategy used in this study aimed at masking Ca^{2+} -dependent InsP_3 catabolism by 3-kinase through the injection of massive amounts of 5-phosphatase, which is not stimulated by Ca^{2+} . In such injected hepatocytes, Ca^{2+} oscillations generated by modest agonist doses are suppressed because

Figure 3. Schematic representation of the interplay between intracellular Ca^{2+} dynamics and InsP_3 metabolism. Dashed arrows indicate positive regulatory feedbacks while the dashed line terminated by a hyphen represents an inhibition. The asterisk indicates that the stimulation of PLC activity by Ca^{2+} only occurs for some isoforms of the enzyme.

of the resulting low level of InsP_3 . At higher doses of agonist, oscillations reappear. Importantly, the characteristics of these oscillations are similar to those of untreated cells at low agonist dose, despite the fact that InsP_3 oscillations due to 3-kinase stimulation by Ca^{2+} do not occur (nearly all the InsP_3 is metabolized by the 5-phosphatase, which is much more abundant because of the injection). This study thus confirms that the oscillations of InsP_3 that would result from Ca^{2+} -regulation of the InsP_3 3-kinase do not play an active role in the control of Ca^{2+} oscillations. Similar conclusions have been reached recently in HSY-EA1 cells, together with the imaging of InsP_3 levels (Tanimura *et al.*, 2009).

Modeling has also been used to define experimental tests that could discriminate between an InsP_3R -based or an InsP_3 metabolism-based mechanism. Sneyd *et al.* (2006) proposed to perturb agonist-induced Ca^{2+} oscillations by the direct, artificial release of InsP_3 in the cytoplasm (flash photolysis of caged InsP_3). In a model where InsP_3 metabolism is at the basis of Ca^{2+} oscillations, this sudden increase in InsP_3 will provoke a delay in the occurrence of the next Ca^{2+} spike, which corresponds to the time required for the level of InsP_3 to go back to its normal range of concentrations during oscillatory cycles. Once this is done, the situation is similar to the prepulse one, and no change in frequency is observed. The situation is drastically different for Ca^{2+} oscillations occurring with a constant level of InsP_3 due to the sequential activation and inhibition of the InsP_3R . In the framework of such a mechanism, the frequency of Ca^{2+} oscillations directly depends on the (constant) level of InsP_3 . Thus, a sudden increase in InsP_3 during agonist-induced Ca^{2+} oscillations provokes a transient rise in frequency.

The interspike interval then progressively decreases to the period of the unperturbed system. The number of spikes necessary to the resettlement of the prepulse periodicity increases with the amount of InsP_3 released into the cell [(Sneyd *et al.*, 2006); see also (Chatton *et al.*, 1998)].

STOCHASTIC ASPECTS OF Ca^{2+} OSCILLATIONS

In experiments, a rise in the level of InsP_3 transforms random, elementary Ca^{2+} increases into regular Ca^{2+} increases propagating as waves in the cytoplasm. This transition can be explained by the fact that the rise in InsP_3 leads to an increase in the number of channels participating in the Ca^{2+} dynamics. The effect of fluctuations is then expected to become rather small, allowing for a deterministic description. To test this hypothesis, we have performed a statistical analysis of the regularity of Ca^{2+} oscillations in noradrenaline-stimulated hepatocytes and found that the coefficient of variation in the period lies between 10% and 15% (Dupont and Combettes, 2009). Stochastic simulations taking into account realistic numbers of InsP_3Rs (about 6000 in a typical hepatocyte) accounted for such a low variability, if the receptors are assumed to be grouped in clusters of a few tens of channels (Dupont *et al.*, 2008; Dupont and Combettes, 2009). This corroborates the idea that repetitive Ca^{2+} spiking can be described as a deterministic oscillator. As the number of clusters is rather low, this oscillator is however perturbed by noise leading to the 10–15% variation in the period. In agreement with this view, this coefficient of variation decreases as the level of InsP_3 rises since the number of active channels increases (Fig. 4). In a small number of cases, very noisy oscillations ($\text{CV} \sim 30\%$) are observed at low levels of stimulation that in most cells do not induce any Ca^{2+} increase. Simulations using Gillespie's algorithm strongly suggest that this situation corresponds to a regime of stochastic resonance. In such a regime, the level of InsP_3 is just below the Hopf bifurcation point (i.e., the threshold that delimitates oscillatory from nonoscillatory states in a fully deterministic regime). As this corresponds to an excitable regime, fluctuations can then sometimes lead the system above the excitation threshold and induce the occurrence of a whole Ca^{2+} spike. Such dynamics lead to highly variable interspike intervals. Both in the simulations and in experiments, this situation is rarely encountered and a slight increase in InsP_3 leads to much more regular Ca^{2+} oscillations, which can be interpreted as a noisy deterministic behavior.

It has also been proposed that Ca^{2+} dynamics are always stochastic. The reason for that would lie in the assumed spatial arrangement of the InsP_3Rs in clusters spaced from each other by several microns. As Ca^{2+} is a poorly diffusible messenger in the cytosol, the Ca^{2+} rise occurring at one puff site would be unable to activate release from adjacent sites. This absence of communication would prevent global signaling. Thus, Ca^{2+} waves could be initiated only if, by chance, a sufficient number of clusters of InsP_3Rs became active at the same time. This process, called nucleation, would lead to a Ca^{2+} increase that is large enough to activate all the InsP_3 -bound InsP_3Rs

Figure 4. Simulations of Ca^{2+} oscillations by a stochastic Gillespie's algorithm taking into account the numbers of Ca^{2+} ions and InsP_3 receptors present in a typical hepatocyte. Both in the model and in experiments, the variability decreases with the period.

and generate a Ca^{2+} spike. Under these circumstances, the variation in the period is of the order of the period itself (Falcke, 2004; Skupin *et al.*, 2008), which is much larger than in noradrenaline-stimulated hepatocytes (Dupont *et al.*, 2008). Interestingly, in this framework, spike initiation—that corresponds to the time required for the concomitant opening of a few adjacent cluster sites—can take very long as it is a random event. This would provide a possible explanation to the long periodicity of Ca^{2+} oscillations as compared to the characteristic times of the InsP_3R dynamics.

CONCLUSIONS

Together with electrophysiology, intracellular Ca^{2+} dynamics is one of the field of cellular physiology where the interplay between experiments and modeling has been the most fruitful. One of the reasons for this is that, as other rhythmic phenomena, they rely on specific, nonlinear feedback processes and can thus hardly be fully approached without mathematical modeling and numerical simulations. In the same manner, cAMP oscillations, circadian rhythms, cell cycle-related variations in the activity of cyclin associated kinases or the tumor-associated p53/mdm2 loop are other oscillatory phenomena in biology whose investigation largely benefits from a modeling approach (Goldbeter, 2008). Given the accessibility of a variety of quantitative data, modeling not only allows the investigation of the molecular mechanisms responsible for Ca^{2+} oscillations but can also lead to a detailed investigation of the cell to cell variability. This can be ascribed to subtle differences in the characteristics of the

three existing isoforms of the InsP_3 receptors that are expressed at different levels in the different cell types. Kinetic characteristics of InsP_3 metabolism also modulate Ca^{2+} oscillations.

Surprisingly, many factors that are known to alter Ca^{2+} oscillations have not yet been deeply considered in models. For example, despite the large number of experimental studies devoted to the mechanism of Ca^{2+} entry (Putney and Bird, 1998), most models for Ca^{2+} oscillations in nonexcitable cells consider a closed system where Ca^{2+} exchanges are limited to fluxes between the cytosol and the ER. Mitochondrial Ca^{2+} handling is also known to alter cellular Ca^{2+} signals in the cytosol (Halestrap, 2009). Although some models have been developed to explain the pumping and releasing properties of suspensions of mitochondria (Selivanov *et al.*, 1998), their implication in intact cells have rarely been investigated theoretically (Marhl *et al.*, 2000; Fall and Keizer, 2001).

Finally, much remains to be done in the field of Ca^{2+} dynamics as to the link between elementary Ca^{2+} events due to the random opening of a few Ca^{2+} channels at low levels of InsP_3 and coordinated, regular Ca^{2+} oscillations. This question pertains to the emergence of a macroscopic self-organized system from a large number of microscopic random entities, which is a central concern in biology. As this transition is observed in experiments just by increasing the level at which the cell is stimulated, Ca^{2+} dynamics can be seen as an ideal system to investigate its physicochemical foundations.

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